

Amendments to the Claims:

This listing of claims replaces all prior versions and listings of claims in the application:

Listing of Claims:

1-15. (cancelled)

16. (currently amended) A method for ~~optimizing~~ modifying simulation or scoring function parameters ~~that utilizes comparisons between designed sequences and natural sequences,~~ the method comprising the steps of:

- a) applying a protein design cycle to produce a variable ~~protein~~ amino acid sequence;
- b) comparing ~~said~~ the variable ~~protein~~ amino acid sequence to at least one natural ~~protein~~ amino acid sequence and/or conformation;
- c) modifying ~~said~~ the simulation or scoring function parameters to reflect ~~said~~ the comparison.

17. (original) A method for optimizing simulation or scoring function parameters that utilizes comparisons between designed sequences and natural sequences, comprising the steps of:

- a) applying a protein design cycle to produce an amino acid probability matrix;
- b) comparing said matrix to at least one natural protein sequence and/or conformation;
- c) modifying said simulation or scoring function parameters to reflect said comparison.

18. (original) A method executed by a computer under the control of a program, said computer including a memory for storing said program, said method comprising the steps of:

- a) inputting a protein backbone scaffold;
- b) applying a first protein design cycle comprising at least one heuristic component to said scaffold to generate at least a first variable sequence;
- c) applying a second protein design cycle comprising at least one heuristic component to said scaffold to generate at least a second variable sequence; and
- d) generating a probability matrix comprising at least said first and second variable sequences.

19 - 31. (cancelled)

32. (original) A method executed by a computer under the control of a program, said computer including a memory for storing said program, said method comprising the steps of:

- a) inputting an ensemble of protein backbone scaffolds;
- b) applying a protein design cycle to each of said scaffolds to generate at least one variable sequence; and
- c) generating a probability matrix comprising a plurality of the variable sequences generated in step b).

33. - 43. (cancelled)

44. (original) A method executed by a computer under the control of a program, said computer including a memory for storing said program, said method comprising the steps of:

- a) inputting at least one protein backbone scaffold;
- b) applying a protein design cycle to generate at least a first variable nucleated state sequence;
- c) sequentially altering in said nucleated state sequence a plurality of amino acids by testing a plurality of rotamers for each amino acid change and calculating the energy of each altered sequence; and
- d) generating a Boltzmann probability matrix comprising a plurality of altered nucleated state sequences.

45 – 47. (cancelled)

48. (new) The method of claim 16 wherein the method is repeated at least once.

49. (new) The method of claim 48 wherein a new backbone structure is selected for each protein design cycle.

50. (new) The method of claim 16 wherein the variable amino acid sequence comprises residue positions that are variable and other residues positions that are fixed.

51. (new) The method of claim 50 wherein, at a fixed residue position, different rotamers are tested during the design cycle.

52. (new) The method of claim 50 wherein active site residues are fixed.

53. (new) The method of claim 16 wherein the variable amino acid sequence is designed to fold to a known structure, and the natural amino acid sequence and/or conformation is/are from a natural protein that has the known structure.

54. (new) The method of claim 16 wherein the simulation or scoring function parameters comprise amino acid reference energies.

55. (new) The method of claim 16 wherein the simulation or scoring function parameters comprise correction factors weighted towards a standard distribution of amino acids.

56. (new) The method of claim 55 wherein the correction factors depend on composition.

57. (new) The method of claim 55 wherein the correction factors depend on structural environment.

58. (new) The method of claim 55 wherein the correction factors depend on secondary structure class.

59. (new) The method of claim 16 wherein the modifying comprises biasing the simulation or scoring function parameters toward the natural amino acid sequence.

60. (new) The method of claim 59 wherein the biasing favors usage of wild-type or homolog residues from the natural amino acid sequence.

61. (new) The method of claim 59 wherein the biasing favors usage of wild-type residues from the natural amino acid sequence.

62. (new) The method of claim 59 wherein the biasing is used for active site residues.
63. (new) The method of claim 16 wherein the modifying comprises biasing the simulation or scoring function parameters away from the natural amino acid sequence.
64. (new) The method of claim 16 wherein the modifying comprises biasing the simulation or scoring function parameters toward a family that includes the natural amino acid sequence.
65. (new) The method of claim 16 further comprising applying the design cycle to produce a plurality of variable amino acid sequences.
66. (new) The method of claim 65 further comprising synthesizing proteins, each comprising a sequence from the plurality of variable amino acid sequences
67. (new) The method of claim 66 further comprising screening the proteins for biological activity.
68. (new) The method of claim 65 further comprising recombining a plurality of the variable amino acid sequences to form additional variable amino acid sequences.
69. (new) The method of claim 16 wherein the protein design cycle comprises a self consistent mean field theory (SCMF) algorithm.
70. (new) The method of claim 16 wherein the protein design cycle comprises a dead end elimination (DEE) algorithm.

71. (new) The method of claim 16 wherein the protein design cycle comprises a genetic algorithm.

72. (new) An article of computer-accessible memory that stores instruction that cause a computer to execute a method comprising:

- a) applying a protein design cycle to produce a variable amino acid sequence;
- b) comparing the variable amino acid sequence to at least one natural amino acid sequence and/or conformation;
- c) modifying the simulation or scoring function parameters to reflect the comparison.